Supporting information

Quantitative MALDI Imaging of Spatial Distribution and Dynamic Changes of Tetrandrine in Multiple Organs of Rats

Content

Peak Normalization

Briefly, the imaging dataset was converted to imzML file and directly imported to MSiReader software (Open Access). The following function is used to normalize the intensity values in the image with the intensity of IS.

$$I_{tetrandrine,x,y}=Max(I_{range(tetrandrine),x,y}) / Max(I_{range(IS),x,y}) \times NormScale$$

For each spectrum, normalization will only be performed if the intensity of the IS peak is above a user defined threshold, NormCutoff (set to 1, default. Minimum intensity value for normalization peak in any given spectrum for normalization to be applied). The mass range for the IS peak is equal to the *m/z* window of tetrandrine (set to ±0.15 Da). The normalized intensity is scaled by the NormScale value (set to 1, default. After normalization, all intensities are multiplied by this value).

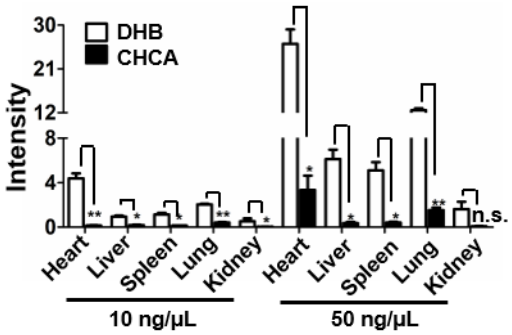

Figure S1. Mean peak intensities of tetrandrine standards (10 ng μL^{-1} and 50 ng μL^{-1}) measured with DHB and CHCA matrices in different tissues. Error bars indicate the standard deviations of triplicates. Significant differences were determined using Student's t-test. Significance levels: *$p < 0.01$ and **$p < 0.005$. n.s. no significant difference.

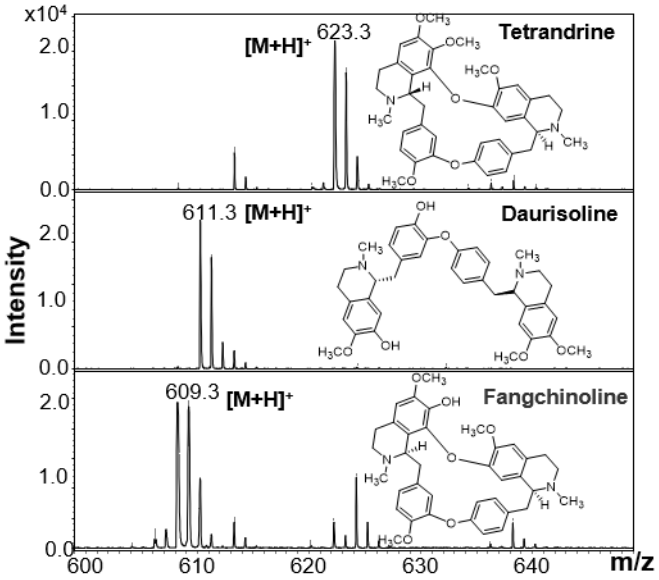

Figure S2. Representative MALDI-TOF mass spectra of tetrandrine, daurisoline (IS) and fangchinoline standard.

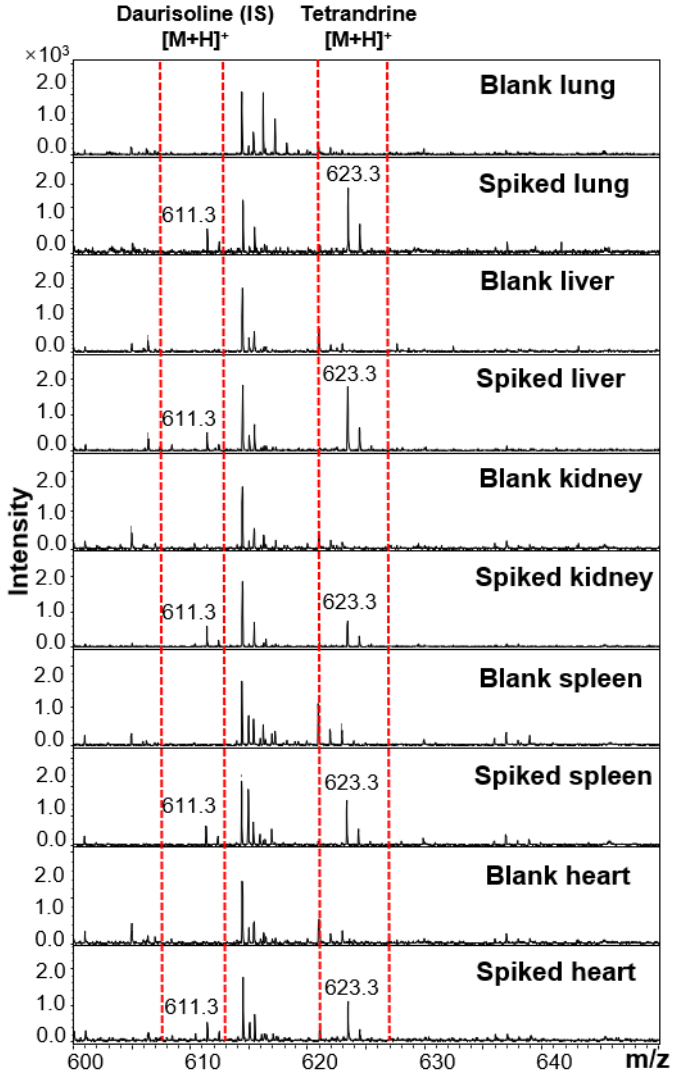

Figure S3. MALDI-TOF mass spectra obtained from various blank tissues and blank tissues spiked with tetrandrine and IS standards.

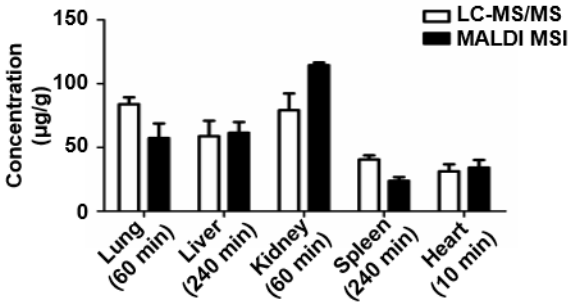

Figure S4. Quantitative results of tetrandrine from representative tissues measured by MALDI MSI and LC-MS/MS. Data was expressed as the mean ± SD from detection of three independent animals.

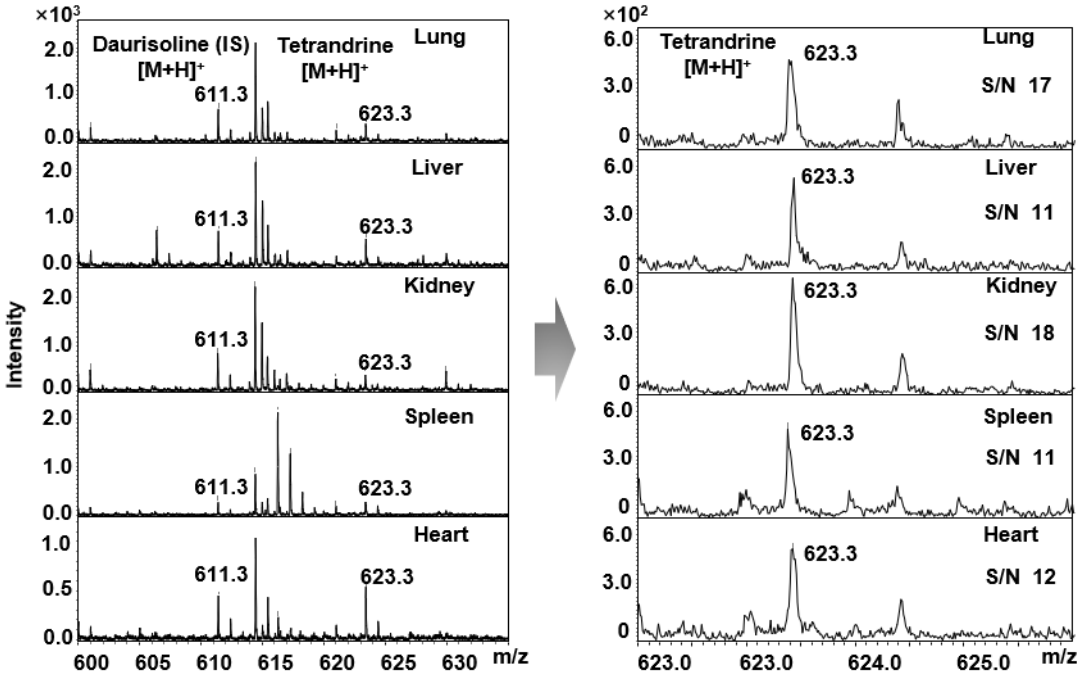

Figure S5. Representative MALDI-TOF MS spectra of tetrandrine spiked with IS in different tissues at lowest limit of quantification level (LLOQ, S/N>10). The zoom (right panel) shows the region of the mass spectra (left panel) in which *m/z* 623.3 should be seen.

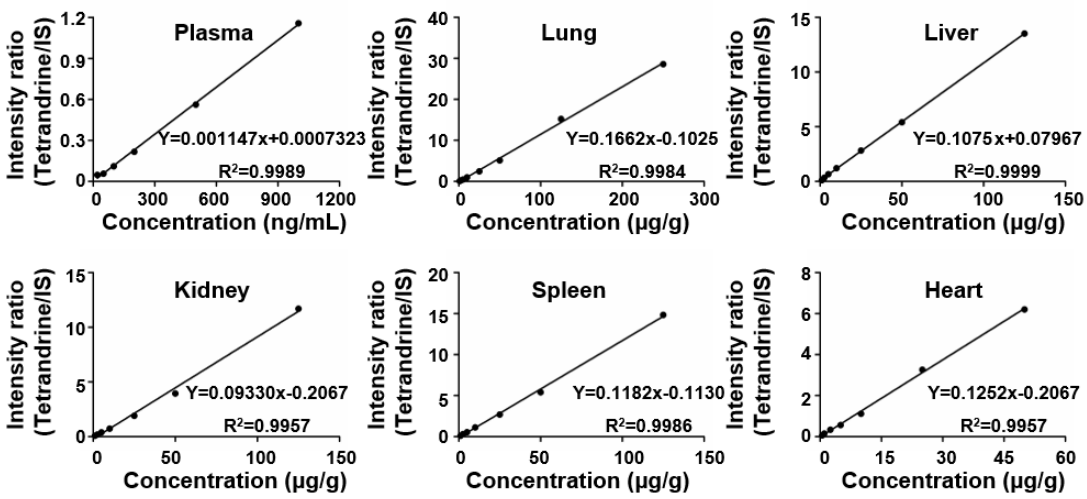

Figure S6. Calibration curves of tetrandrine quantified by LC-MS/MS analysis of plasma, lung, liver, kidney, spleen and heart samples.

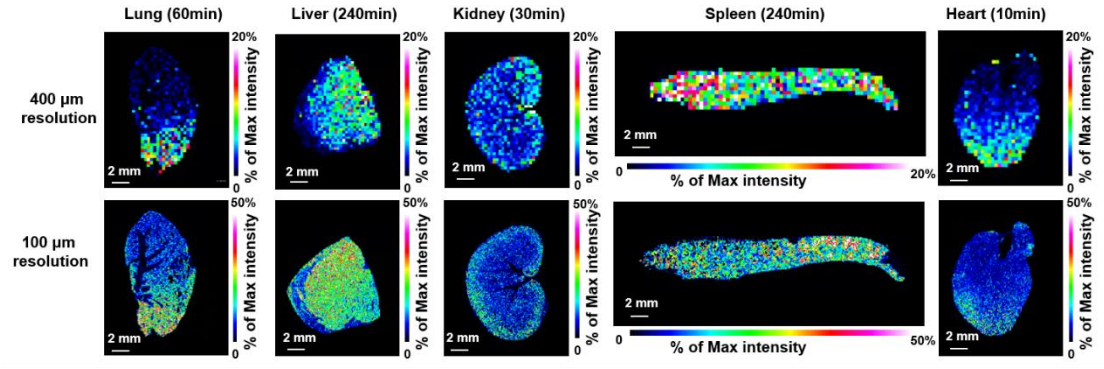

Figure S7. Representative MS images of tetrandrine in different tissues acquired at 400 µm (upper) and 100 µm (lower) step size.

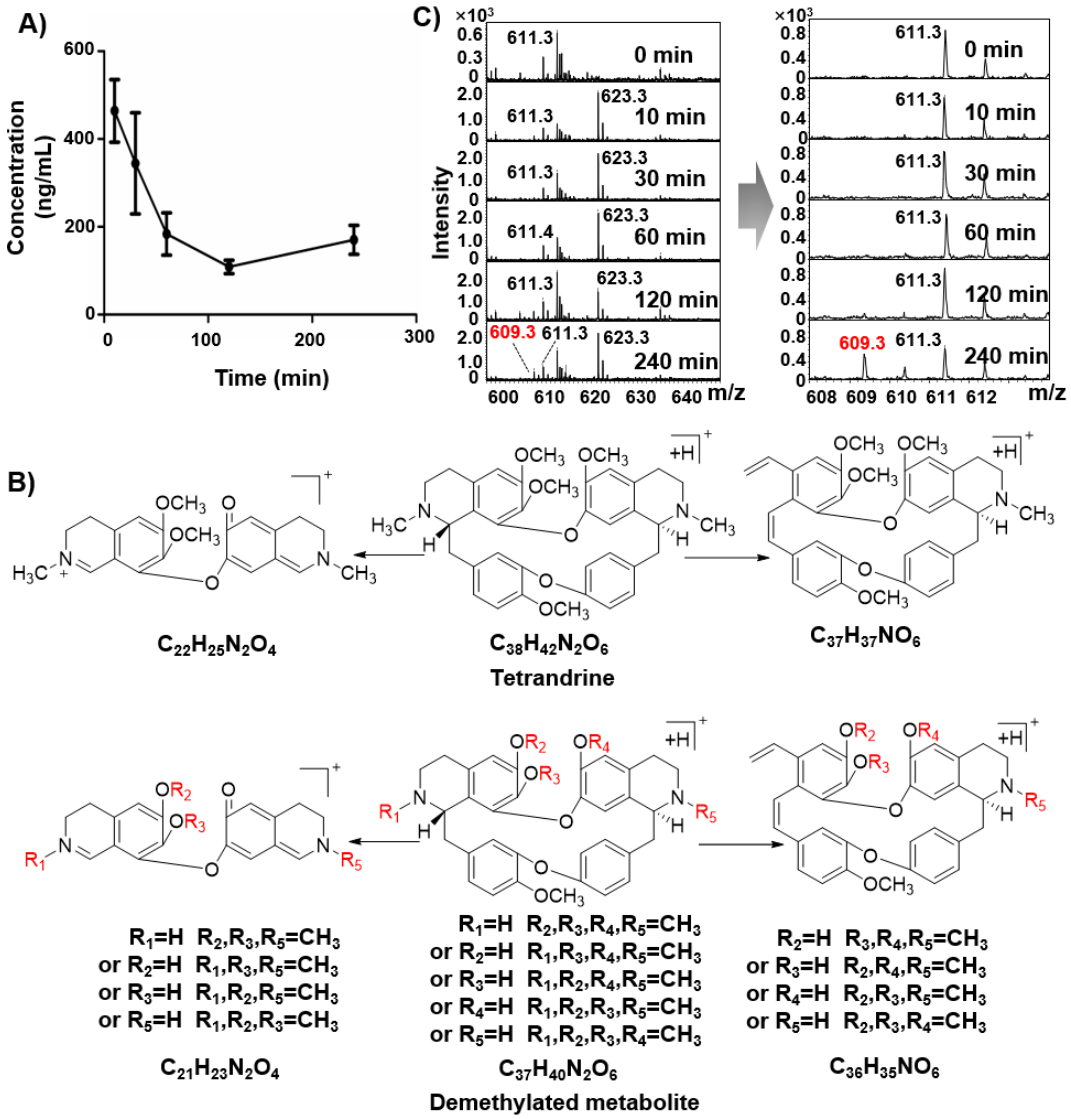

Figure S8. A) Time-concentration curve of tetrandrine in plasma determined by LC-MS/MS analysis. B) Fragmentation pathway of tetrandrine and its demethylated metabolite; C) Representative MALDI-TOF MS spectra of tetrandrine demethylation metabolite (*m/z* 609.3) in liver tissue at 0, 10 min, 30 min, 60 min, 120 min and 240 min post-dose. The zoom (right panel) shows the region of the mass spectra (left panel) in which *m/z* 609.3 should be seen.

Table S1 LLOQ of tetrandrine and linearity of calibration curves obtained by using non-normalization and IS-based normalization in different tissues (*n=3*).

	LLOQ ($ng\ \mu L^{-1}$)	Concentration ($ng\ \mu L^{-1}$)	Non-normalization		IS-based normalization	
			Linear equations	R^2	Linear equations	R^2
Lung	3	5-200	y=(0.04626 ±0.002881)x+(0.9790±0.2709)	0.9847	y=(0.3185±0.01389)x-(1.406±1.306)	0.9924
Liver	4	5-200	y=(0.05030 ±0.002329)x+(0.03618 ±0.2190)	0.9915	y=(0.03123±0.0007597)x-(0.09898±0.07142)	0.9976
Kidney	4	5-200	y=(0.06401±0.01004)x+(1.708±0.9437)	0.9104	y=(0.03641±0.0001532)x+(0.2950±0.1440)	0.9930
Spleen	4	5-200	y=(0.06670 ±0.001706)x+(0.2899±0.1604)	0.9974	y=(0.06529±0.0009840)x+(0.03789±0.09250)	0.9991
Heart	4	5-200	y=(0.09242±0.005539)x+(0.2176±0.5207)	0.9858	y=(0.02624±0.001084)x+(0.2054±0.1019)	0.9932